International Journal of Medicine and Pharmaceutical Sciences (IJMPS)
ISSN(P): 2250-0049; ISSN(E): 2321-0095
Vol. 4, Issue 2, Apr 2014, 85-90

TRANS STELLAR • Journal Publications • Research Consultancy

NOVEL ANTIBACTERIAL COMBINED AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

HABEEB S. NAHAR[1] & ANWAR KADHIM AL-SAFFAR[2]

[1]Department of Microbiology, College of Sciences, Babylon University, Iraq

[2]Assistant Professor, Department of Microbiology, College of Medicine, Babylon University, Iraq

ABSTRACT

Background: *Staphylococcus aureus* is an opportunistic pathogen often carried asymptomatically on the human body. Methicillin-resistant *S. aureus* (MRSA) includes those strains that have acquired a gene giving them considerable resistance to first line of treatment, methicillin and essentially to all other beta-lactam antibiotics. MRSA was first reported in United Kingdom on 1961 as a serious human pathogen and currently becomes resistant to almost all antibiotics, therefore treatment can be challenged.

Aim: This study was suggested and designed to evaluate the antibacterial effect of a new compound of Sidr extract (*Ziziphusspina-christi-L,var. inermis Boiss*) combined with Hydrogen peroxide (H2O2) against MRSA.

Methods: An aqueous extract of sidr leaves was prepared (62. 5 g/L.) under suitable laboratory conditions (time, temperature and others necessary conditions). Hydrogen Peroxide (1.5%) was also prepared. MRSA isolates obtained from the Central Laboratory in Hilla, Babylon province. Those isolates have preliminary characterized as Methicillin (10 mg.) and Oxacillin (10 mg.) resistant but relatively sensitive to Vancomycin (10 mg.). The isolates were reidentified in our Lab. for confirmation using the diagnostic tests specific for MRSA. MRSA isolates were treated by an **appropriate concentration** (**the knowhow**) of a mixture included an aqueous extract of Sidr leaves (62. 5 g./L.) and Hydrogen peroxide (1.5%) using wells in agar method.

Results: The results indicated that MRSA isolates investigated in this study revealed fully resistance towards both Methicillin and Oxacillin (no an inhibition zones were seen after 24 hrs. incubation at 37C°) while these isolates exhibited a remarkable sensitivity towards Vancomycin (10 mg.) and the prepared combined, since the diameter of inhibition zone was 22 mm. and 21 mm. respectively.

KEYWORDS: Novel Antibacterial Combined, MRSA, Sidr Extract, H2O2

INTRODUCTION

Staphylococcus aureus frequently produces an enzyme known as *B*-lactamase which makes the organism resistant to almost all Beta-lactams antibiotics, e.g. penicillins and sephalosporins[1.] MRSA cause a serious problem in hospitals, nursing homes, and other health care settings[2]. These organisms are capable to produce various toxic products resulting in multiform diseases. Hospitals- acquired MRSA (HAMRSA) strains have caused diseases, including severe sepsis and pneumonia[3]. Virtually, all MRSA produce an additional penicillin-binding protein, which confers resistance to all currently available *B*-lactam agents and also resistant to penicillinase-stable penicillin such as Oxacillin[4].

Methicillin and Oxacillin resistant staphylococci carry the *mecA* which is responsible for this resistance[5].

MRSA infections are traditionally associated with exposure to a health care environment, especially the inpatient hospital setting HA-MRSA[6]. MRSA was first reported in 1961, two years after the introduction of Methicillin for treatment of penicillin-resistant *S. aureus* infections[7].

MRSA infections have become increasingly common over the last several decades and are now present or endemic Worldwide, and increasing proportion of MRSA isolates were from hospitalized patients admitted from the community[8].

MRSA is a particularly difficult problem due to the emergence of resistance of resistant strains. Many hospital- acquired MRSA strains are only susceptible to vancomycin, thus, there are strong concerns about the possible development and spread of vancomycin resistance in MRSA[9].

Ziziphusspina-christi commonly known as Christ's Thorn Jujube, is a deciduous tree and native to the warm-temperate and subtropical regions, including North Africa, South Europe, Mediterranean, Australia, tropical America, South and East of Asia and Middle East[10]. It belongs to the Rhamnaceae family in the order of Rosales that contains about 60 genera and more than 850 species. The genus Ziziphus consists of about 100 species of deciduous or evergreen trees and shrubs throughout the world[11].

The Ziziphusspina- Christi was known to be active against wide spectrum of bacteria due to presence of betulic and cyanotic acid, three cyclopeptide alkaloids as well as four saponim glycosides and several flavonoids[12].

MATERIALS AND METHODS

Preparation of the Sidr tree-*Ziziphusspina-christi (L) var. inermisBoiss*, The fresh sidr leaves after being weighed as 62.5 g/L. There was cleaned from the dust through washing in tap water. One liter of distilled water was added to each sample and heated up to boiling. The solution was left in the refrigerator for 12 hours .the remnants of the leaves were discarded, while the liquids extract was mixed with 1.5% hydrogen peroxide to get the new invited antibacterial

Disc diffusion test: the Kirby-bauer method is a standardized system that takes all variables into consideration. It is sanctioned by the United States FDA and the Subcommittee on Antimicrobial Susceptibility Testing of the NCCLS[13].

It was performed by using a pure culture of previously identified bacterial organism. The inoculum to be used in this test was prepared by adding growth from 5 isolated colonies grown on blood agar plates to 5 ml of nutrient Broth, this culture was then incubated for 2 hrs. To produce a bacterial suspension of moderate turbidity that compared with turbidity of ready-made (0.5) McFarland tube standard provided by Biomerieux/ France. A sterile swap was used to obtain an inoculum from the standardized culture, this inoculum was then swapped on Mueller-Hinton plate.

2-The antibiotic discs were placed on the surface of the medium at evenly spared intervals with flamed forceps, then incubated at 33C to 35C for a full 24 hrs. before reading the results to identify cells expressing heteroresistance[14]. Worthily for mentioning, the incubation period were modified to 16-18 hrs. When methicillin(10mcg), oxacillin (10mcg) and vancomycinused (10mcg) as indicated by according[15] .

3-Antibiotics inhibition zones were measured using a transparence ruler. Zone size was compared to standard zones from the[15], to determine the susceptibility of organism to each antibiotics.

Well diffusion method: In this method, Muller- Hinton agar plate was prepared by equally cutting spaced well (6mm), then the plates were inoculated with cotton swab dipping into scrow tube containing bacterial suspension and streaked over the surface of plates. After that, Muller-Hinton agar wells were filled with 0.1 ml of prepared concentrations for each mixture (sidr extract 62.5 g/L and 1.5% hydrogen peroxide) and incubated the plates at 37 C for 24 hr. The susceptibility to there mixer was determined by measuring the inhibition zone around the wells for each concentration[16].

RESULTS AND DISCUSSIONS

Resistance of Gram positive bacteria continues to be an important clinical therapeutic problem, such that can be found in an increasing multidrug resistance in MRSA[14]. Different strains of *S.aureus* become strongly resistant to methicillin. These strains are referred to as methicillin resistant *S. aureus* (MRSA). The MRSA was recently emerged worldwide as the major nosocomial pathogen and most important strains of *S.aureus* causing severe and relatively most serious infections particularly in hospitalized patients. some strains of *S. aureus*are resistant to the *B*-lactamase- resistant penicillins, by virtue of changes in the penicillin-binding protein in their cell membrane[17].

In this study, MRSA isolates exhibited high resistance rate toward methicillin and oxacillin . Staphylococcal resistance to either Methicillin or oxacillin occurs when the organism including an altered PBP (PBP2A) that is coded by the *mecA* gene. Conventional susceoptibility tests of *S. aureus*have been performed for the detection of resistance to oxacillin (10mcg) and methicillin (10mcg) by disc diffusion test according to the standard method recommended by (18), documentations. And vancomycin(10mcg) inhibition zone of sensitive isolate in disc diffusion test was determined at 22mm according to[18]. Accordingto[19], who found resistance of MRSA to vancomycin because of resistance genes e.g*van*A, *van*B, *van*C1, *van*C2, and / or *van*C3 genes.(Table 1)

Table 1: The Sensitivity of MRSA to the Antibiotics

Strain	Antibiotics Susceptibility		
	Methicillin (10mcg)	Oxacillin (10mcg)	Vancomycin (10mcg)
S. aureus (MRSA)	R	R	(S) 22mm

R: resistance. **S**: sensitive.

Hydrogen peroxide (HP) is an active agent that affects a wide range of organisms such as bacteria, yeast, fungi, viruses, and spores[20]. *Z. spina-christi* has recently been shown to have antibacterial, antifungal and antioxidant activities[21].

In this study (Table 2), the result that MRSA isolate was sensitive to mixture (sidr extract 62.5% and 1.5% hydrogen peroxide) inhibition zonein well diffusion test was determined at 21mm according to[18].While the MRSA isolate was resistance to each another antibacterial solutions sidr extract 62.5 g/L and hydrogen peroxide 1.5%.

Table 2: The Sensitivity of MRSA to the Antibacterial Solutions

Strain	Antibacterial Solutions		
	Sidr Extract 62.5 g/L	Hydrogen Peroxide 1.5%	Mixture (Sidr Extract 62.5% with Hydrogen Peroxide 1.5%)
S. aureus(MRSA)	**R**	**R**	**(S) 21mm**

The mixture of the sidr extract 62.5% with 1.5% hydrogen peroxide was effective of to inhibit completely MRSA**.**

In this study we assessed the antibacterial effect of special solution made by a mixture of Sidr extract with Hydrogen peroxide.

REFERENCES

1. Ravel, R. (1995). Clinical laboratory medicine: Clinical applications of laboratory data. 6th ed. Mc. Graw-Hill Companies.PP:111-117.
2. Bronw, D.; Edwards, D. I.; Hawkey, P. M.; Morrison, D. and Ridgway, G. L. (2005).Guidelines for the laboratory diagnosis of and susceptibility testing of methicillin- resistant *Staphylococcus aureus* (MRSA).J. Antimicrobchemother., **56**: 1000-1018.
3. Bratu, S.; Eramo, A.; Kopec, R.; Coughlin, E.; Ghitan, M.; Yost, R.; Chapnick, E. K.; Landman, D. and Quale, J. (2005). Community-associated methicillin-resistant *Staphylococcusaureus* in hospital nursery and maternity units. Emerg. Infect. Dis. **11**:808-813.
4. Brown, D.F. (2001). Detection of methicillin/ oxacillin resistance in staphylococci. J. Antimicrob. Chemother., **48**: 65-70.
5. Louie, L.; Matsumura, S.O.; Choi, E.; Louie, M. and Simor, A. E. (2000).Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus***.** J. Clin. Microbiol., **38**:2170-2173.
6. Enright, M. C.; Robinson, D. A. and Randle, G. (2002). The evolutionary history of methicillin- resistant *Staphylococcusaureus* (MRSA). Proc.Natl. Acad. Sci. USA., **99**: 7687-7692.
7. Neu, H. C. (1992). The crisis in antibiotic resistance. J. Science, **257**:1064-1073.
8. Voss, A.; Loeffen, F.; Bakker, J.; Klaassen. C. and Wulf, M. (2005). Methicillin-resistant Staphylococcus aureus in farming pig. Emerg. Infect. Dis., **11**:1965-1966.
9. Murray, P.R.; Baron, E. J.; Jorgensen, J. H.; Pfaller, M. A. and Yolen, R. H. (2003). Manual of clinical microbiology. 8th. Ed., American Society for Cicrobiology, Washington, DC., Vol. (1).
10. Yossef, H. E.; Khedr, A.A. andMahran. M.Z. (2011). Hepatoprotective activity and antioxidant effects of El Nabka (*Zizyphusspina-christi*) fruits on rats hepatotoxicity induced by carbon tetrachloride. Nat. Sci. **9**:1-7.
11. Abalaka, M.E.; Daniyan, S.Y.and Mann, A. (2010). Evaluation of the antimicrobial activities of two *Ziziphus*species (*Ziziphusmauritiana*L. and *Ziziphusspina-christi*L) on some microbial pathogens. Afr. J. Pharm. Pharmacol. **4**:135-139.
12. Asgarpanah, J. and Haghighat, E. (2012). Phytochemistry and pharmacologic properties of *Ziziphusspinachristi* (L.) Willd. African Journal of harmacy and Pharmacology Vol. **6**, pp. 2332-2339.
13. Benson, H. J. (2001). Microbiological application, laboratory manual in general microbiology. 8thed. W.B. Mcgraw Hill.
14. Weigelt, J. A (2007). MRSA Informa Healthcare. USA, Inc. Wertheim, H.F.; Melles, D.C.; Vos, M.C; (2005). The role of nasal carriage in *Staphylococcusaureus* infections. Lancet. Infect. Dis. **5**:751-762.

15. Clinical and Laboratory Standards Institute (CLSI) (2007). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S17. Vol. **27**, No. 1. National committee for clinical Laboratory Standards, Wayne, PA.USA.

16. Hangnga, V. U.; Lana, F.; Washington, J.; Dang, T.; Villarreal, C. *et al*. (2002). Burn Wound Infection Susceptibilities to Topical Agents: the Nathan's Agar Well Diffusion Technique.

17. Levinson (2008). Reviewof medical microbiology and immunology: examination and board review, 10th. ed. McGraw-Hill Companies, Inc.; ch. 15.

18. Clinical and Laboratory Standards Institute consensus (CLSI) (2010). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S20. Vol. 30, No. 1. National committee for clinical Laboratory Standards, Wayne, PA.USA.

19. Tenover,F. C. ; Lancaster, M. V. and Hill, B. C. (1998). Characterization of *Staphylococci* with reduced susceptibilities to vancomycin and other glycopeptides. J. Clin. Microbiol. **36**: 1020-1027.

20. Hidron, A. I.; Edwards, J.R. and Patel, J. *et al*. (2008). Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Annual Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease. Infect Control HospEpidemiol; **29**:996-1011.

21. Dangoggo, S.M; Hassan, L.G.; Sadiq, I.S and Manga, S.B (2012). Photochemical Analysis and Antibacterial Screening of Leaves of DiospyrosMespiliformis and ZiziphusSpina-Christi. J. Chemical Engen. 1: 31-37.